

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (previously presented) A method for identifying one or more low abundance sequences differing by one or more single-base changes, insertions, or deletions from a high abundance sequence, in a sample containing a plurality of target nucleotide sequences comprising:

providing a sample potentially containing one or more low abundance target nucleotide sequences with at least one sequence difference each from the high abundance target sequences;

providing a primary oligonucleotide primer set characterized by (a) a first oligonucleotide primer containing a target-specific portion, and (b) a second oligonucleotide primer containing a target-specific portion, wherein the primary oligonucleotide primers hybridize to complementary strands of high and low abundance target nucleotide sequences to permit formation of a polymerase chain reaction product, but have a mismatch which interferes with formation of such a polymerase chain reaction product when hybridized to any other nucleotide sequence present in the sample;

providing a polymerase;

blending the sample, the primary oligonucleotide primers, and the polymerase to form a primary polymerase chain reaction mixture;

subjecting the primary polymerase chain reaction mixture to two or more polymerase chain reaction cycles;

providing a secondary oligonucleotide primer set characterized by (a) a first oligonucleotide primer, having a target-specific portion and a 5' upstream secondary primer-specific portion, and (b) a second oligonucleotide primer, having a target-specific portion and a 5' upstream secondary primer-specific portion, wherein the secondary oligonucleotide primers in a particular set hybridize to complementary strands of the primary extension products to permit formation of a secondary polymerase chain reaction product which contains or creates a restriction endonuclease recognition site when amplifying the high abundance target, but does not contain or create a restriction endonuclease recognition site when amplifying the one or more low abundance targets;

providing a polymerase;

blending the primary extension products, the secondary oligonucleotide primers, and the polymerase to form a secondary polymerase chain reaction mixture;

subjecting the secondary polymerase chain reaction mixture to two or more polymerase chain reaction cycles, wherein high abundance secondary extension products contain a restriction site but low abundance secondary extension products do not;

providing a restriction endonuclease;

blending the secondary extension product and the restriction endonuclease to form an endonuclease digestion reaction mixture;

subjecting the endonuclease digestion reaction mixture to an endonuclease digestion reaction such that the restriction endonuclease recognizes and cleaves the restriction endonuclease recognition site contained within or created when amplifying the high abundance target but not the low abundance target in the secondary extension products, thus selectively destroying the high abundance secondary extension products;

providing a tertiary oligonucleotide primer set characterized by (a) a first tertiary primer containing the same sequence as the 5' upstream portion of the first oligonucleotide primer of the secondary oligonucleotide primer set, and (b) a second tertiary primer containing the same sequence as the 5' upstream portion of a second oligonucleotide primer of the secondary oligonucleotide primer set, wherein the set of tertiary oligonucleotide primers are amplification primers for amplification of all the secondary extension products;

blending the secondary extension products, the tertiary oligonucleotide primer set, and the polymerase to form a tertiary polymerase chain reaction mixture;

subjecting the tertiary polymerase chain reaction mixture to two or more polymerase chain reaction cycles;

providing a plurality of oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe, having a tertiary extension product-specific portion and a detectable reporter label, and (b) a second oligonucleotide probe, having a tertiary extension product-specific portion, wherein the oligonucleotide probes in a particular set ligate together when hybridized adjacent to one another on a complementary tertiary extension product-specific portion, but have a mismatch which interferes with said ligation when hybridized to any other nucleotide sequence present in the sample;

providing a ligase;

blending the tertiary extension product, the plurality of oligonucleotide probe sets, and the ligase to form a ligase detection reaction mixture;

subjecting the ligase detection reaction mixture to one or more ligase detection reaction cycles comprising a denaturation treatment, wherein any hybridized oligonucleotides

are separated from the tertiary extension products, and a hybridization treatment, wherein the oligonucleotide probe sets hybridize at adjacent positions in a base-specific manner to their respective tertiary extension products, if present, and ligate to one another to form a ligation product sequence containing (a) the detectable reporter label and (b) the tertiary extension product-specific portions connected together, wherein the oligonucleotide probe sets may hybridize to nucleotide sequences other than their respective complementary tertiary extension products but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment; and

detecting the reporter labels of the ligation product sequences, thereby identifying the presence of one or more low abundance target nucleotide sequences in the sample.

2. (previously presented) A method according to claim 1, wherein the oligonucleotide probes in an oligonucleotide probe set have a unique length whereby the ligation product sequences which they form are distinguished from other ligation product sequences, said method comprising:

separating the ligation product sequences by electrophoretic mobility prior to said detecting and

distinguishing, after said detecting, the ligation product sequences which differ in electrophoretic mobility.

3. (previously presented) A method according to claim 1, wherein the second oligonucleotide probe of each oligonucleotide probe set further comprises an addressable array-specific portion, said method further comprising:

providing a solid support comprising an array of address-specific capture oligonucleotides, and

contacting the ligase detection reaction mixture, after said subjecting it to one or more ligase detection reaction cycles, with the solid support to hybridize the ligation product sequences to the capture oligonucleotides in a base-specific manner, thereby capturing the addressable array-specific portions to the solid support at the site with the complementary capture oligonucleotide, wherein said detecting identifies the presence of ligation product sequences captured using the addressable array-specific portions and immobilized to the solid support at particular sites, thereby identifying the presence of one or more target nucleotide sequences in the sample.

4. (previously presented) A method according to claim 1 further comprising:

quantifying the amount of the low abundance sequence, wherein said quantifying comprises:

providing a known amount of one or more marker target nucleotide sequences as an internal standard;

providing one or more internal standard sequence-specific oligonucleotide probe sets specifically designed for hybridization to the internal standard, wherein the internal standard sequence-specific oligonucleotide probe sets have (1) a first oligonucleotide probe with a target-specific portion complementary to the marker target nucleotide sequence, and (2) a second oligonucleotide probe with a target-specific portion complementary to the marker target nucleotide sequence and a detectable reporter label;

blending the internal standard, and the internal standard sequence-specific probe sets, with the ligase detection reaction mixture; and

quantifying the amount of ligation product sequences by comparing the amount of ligation product sequences generated from the unknown low abundance sample to the amount of ligation product sequences generated from said internal standard to provide a quantitative measure of one or more low abundance target nucleotide sequences in the sample.

5. (previously presented) A method according to claim 4, wherein one or more low abundance sequence is present in less than a 1:1,000 molar ratio relative to the amount of the high abundance sequence present in the sample.

6. (previously presented) A method according to claim 4, wherein one or more low abundance sequence is present in less than a 1:10,000 molar ratio relative to the amount of the high abundance sequence present in the sample.

7. (previously presented) A method according to claim 4, wherein one or more low abundance sequence is present in less than a 1:100,000 molar ratio relative to the amount of the high abundance sequence present in the sample.

8. (currently amended) A method according to claim 1, wherein prior to providing the secondary oligonucleotide primer set, said method comprises:

providing a pre-secondary oligonucleotide primer set characterized by (a) a first oligonucleotide primer, having a target-specific portion, and (b) a second oligonucleotide primer, having a target-specific portion, wherein the target-specific portions are identical or substantially identical to the secondary oligonucleotide primer set but at least one primer contains one or more nucleotide analogs, wherein the oligonucleotide primers in a particular pre-secondary oligonucleotide primer set hybridize to complementary strands of the primary extension products to form a pre-secondary polymerase chain reaction product which contains one or more nucleotide analogs and opposite strand base changes, wherein the pre-secondary oligonucleotide primer set facilitates conversion of the primary polymerase chain reaction product sequence into a restriction endonuclease recognition site in the subsequent secondary polymerase chain reaction;

providing a polymerase;

blending the primary extension products, the pre-secondary oligonucleotide primers, and the polymerase to form a pre-secondary polymerase chain reaction mixture;

subjecting the pre-secondary polymerase chain reaction mixture to two or more polymerase chain reaction cycles comprising a denaturation treatment, wherein hybridized nucleic acid sequences are separated, a hybridization treatment, wherein the pre-secondary oligonucleotide primers hybridize to the primary extension products, an extension treatment, wherein the hybridized pre-secondary oligonucleotide primers are extended to form pre-secondary extension products complementary to the primary extension products, wherein the pre-secondary extension products contain one or more nucleotide analogues and opposite strand base changes which facilitate conversion of the primary polymerase chain reaction product sequence into a restriction endonuclease recognition site in the subsequent secondary polymerase chain reaction, wherein the pre-secondary extension products are then used in place of the primary extension products in the secondary polymerase chain reaction mixture, whereby the efficiency and accuracy of converting the high abundance primary polymerase chain reaction product into a secondary polymerase chain reaction product containing a restriction endonuclease site is improved.

9. (previously presented) A method according to claim 8, wherein the oligonucleotide probes in an oligonucleotide probe set have a unique length whereby the ligation product sequences which they form are distinguished from other ligation product sequences, said method further comprising:

separating the ligation product sequences by electrophoretic mobility prior to said detecting and

distinguishing, after said detecting, the ligation product sequences which differ in electrophoretic mobility.

10. (previously presented) A method according to claim 8, wherein the second oligonucleotide probe of each set further comprises an addressable array-specific portion, said method further comprising:

providing a solid support comprising an array of address-specific capture oligonucleotides and

contacting the ligase detection reaction mixture, after said subjecting it to one or more ligase detection reaction cycles, with the solid support to hybridize the ligation product sequences to the capture oligonucleotides in a base-specific manner, thereby capturing the addressable array-specific portions to the solid support at the site with the complementary capture oligonucleotide, wherein said detecting identifies the presence of ligation product sequences captured using the addressable array-specific portions and immobilized to the solid support at particular sites, thereby identifying the presence of one or more target nucleotide sequences in the sample.

11. (previously presented) A method according to claim 8 further comprising:

quantifying the amount of low abundance sequence, wherein said quantifying comprises:

providing a known amount of one or more marker target nucleotide sequences as an internal standard;

providing one or more internal standard sequence-specific oligonucleotide probe sets specifically designed for hybridization to the internal standard, wherein the internal standard sequence-specific oligonucleotide probe sets have (1) a first oligonucleotide probe with a target-specific portion complementary to the marker target nucleotide sequence, and (2) a second oligonucleotide probe with a target-specific portion complementary to the marker target nucleotide sequence and a detectable reporter label;

blending the internal standard and the internal standard sequence-specific probe sets with the ligase detection reaction mixture; and

quantifying the amount of ligation product sequences by comparing the amount of ligation product sequences generated from the unknown low abundance sample to the amount of ligation product sequences generated from said internal standard to provide a

quantitative measure of one or more low abundance target nucleotide sequences in the sample.

12. (previously presented) A method according to claim 11, wherein one or more of a low abundance sequence is present in a molar ratio of than less than 1:1,000 relative to the amount of the high abundance sequence in the sample.

13. (previously presented) A method according to claim 11, wherein one or more of a low abundance sequence is present in a molar ratio of than less than 1:10,000 relative to the amount of the high abundance sequence in the sample.

14. (previously presented) A method according to claim 11, wherein one or more of a low abundance sequence is present in a molar ratio of than less than 1:100,000 relative to the amount of the high abundance sequence in the sample.

15. (original) A method according to claim 8, where the nucleotide analog of at least one oligonucleotide primer of the pre-secondary oligonucleotide primer set is at the 3' end of the primer.

16. (original) A method according to claim 8, where the nucleotide analog is selected from the group consisting of 1-(2'-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide, 1-(2'-deoxy-β-D-ribofuranosyl)-3-nitropyrrole, 2'-deoxyinosine, 6-(2'-deoxy-β-D-ribofuranosyl)-6H,8H-3,4-dihydropyrimido[4,5-c][1,2]oxazine-7-one, 2-amino-7-(2'-deoxy-β-D-ribofuranosyl)-6-methoxyaminopurine, 1-(2'-deoxy-β-D-ribofuranosyl)-4-iodopyrazole, 1-(2'-deoxy-β-D-ribofuranosyl)pyrrole-3-carboxamide, and 1-(2'-deoxy-β-D-ribofuranosyl)-4-nitropyrazole.

17. (previously presented) A method according to claim 1 further comprising:

blending the ligation product sequences and the restriction endonuclease, wherein the restriction endonuclease recognizes and cleaves the restriction endonuclease recognition site contained within any remaining high abundance target, thereby selectively destroying the high abundance tertiary extension products.

18.-22. (canceled)